Data Evaluation Record on the Toxicity of Flufenacet Technical to Sheepshead Minnow (*Cyprinodon variegatus*), Early Life Cycle

PMRA Submission Number {.....} EPA MRID Number 49244201

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	416447
	OECD Data Point	{.....}
	EPA MRID	49244201
	EPA Guideline	850.1400

Test material: Flufenacet Technical **Purity:** 98.83%
Common name Flufenacet
Chemical name: IUPAC: 4′-fluoro-*N*-isopropyl-2-[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yloxy]acetanilide
CAS: *N*-(4-fluorophenyl)-*N*-(1-methylethyl)-2-[[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]acetamide
CAS No.: 142459-58-3
Synonyms: None reported

Primary Reviewer: Christie E. Padova **Signature:** Christie E. Padova
Staff Scientist, CSS-Dynamac Corporation **Date:** 02/19/14

Secondary Reviewer: John Marton, Ph.D. **Signature:**
Environmental Scientist, CDM Smith **Date:** 09/25/14

Primary Reviewer: Geoffrey Sinclair, Biologist **Date:** 11/20/14
{EPA/OECD/PMRA}

Secondary Reviewer(s): {.....} **Date:** {.....}
{EPA/OECD/PMRA}

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category	{.....}	[For PMRA]
EPA PC Code	121903	

Date Evaluation Completed: {dd-mm-yyyy}

CITATION: Banman, C.S., T.M. Alexander and S. Moore. 2013. Early Life Stage Toxicity of Flufenacet Technical to the Sheepshead Minnow (*Cyprinodon variegatus*) Under Flow-through Conditions. Unpublished study performed by SynTech Research Laboratory Services, LLC, Stilwell, KS. Laboratory Project ID: EBFOL244. Study sponsored by Bayer CropScience, Research Traingle Park, NC. Study initiated May 24, 2013 and completed August 14, 2013.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the toxicity of a pesticide to fish, early life cycle. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

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EXECUTIVE SUMMARY:

The 35-day chronic toxicity of flufenacet to the early life-stage of sheepshead minnow (*Cyprinodon variegatus*) was studied under flow-through conditions. Fertilized eggs/embryos (140/level, 24- to 48-hours old) were exposed at nominal concentrations of 0 (negative control), 50, 100, 200, 400 and 800 µg ai/L. Mean-measured concentrations were <4.0 (<LOQ, control), 49, 95, 174, 339 and 677 µg ai/L, respectively. Fish were thinned (80/level) on Day 6. The test system was maintained at 24.7 to 25.5°C and a pH of 8.1 to 8.2. The overall NOAEC and LOAEC were 49 and 95 µg ai/L, respectively, based upon a treatment-related reduction in standard length and dry weight on Day 35 at the ≥95 µg ai/L levels.

No treatment-related effect on survival was indicated at any level: alevin survival (Day 6) ranged from 87.9 to 90.7% for all levels and fry survival (Day 35) ranged from 95.0 to 98.8%. No treatment-related effect on time to hatch was observed. On Days 5 and 6, percent hatch for all levels ranged from 29.3 to 39.3% and 84.3 to 89.3%, respectively, no statistically-significant differences from the control indicated. At the 677 µg ai/L level, fish were observed to be swimming at the bottom of the test vessel, except when being fed, beginning on Day 32.

Growth (at study termination) was the most sensitive endpoint. For the mean-measured 0 (control), 49, 95, 174, 339 and 677 µg ai/L treatment levels, standard lengths averaged 19.4, 19.3, 19.0, 18.8, 18.6 and 18.1 mm, respectively, and dry weights averaged 62.7, 61.9, 58.1, 58.8, 56.9 and 50.8 mg, respectively. For both growth parameters, differences were statistically-significant compared to the control (p=0.05) at the ≥95 µg ai/L levels.

This study is scientifically sound and meets the guideline requirements for an early life stage toxicity study with fish. It is therefore classified as acceptable.

Results Synopsis

Test Organism Size/Age (mean Weight or Length): Embryos, 24- to 48-hours old
Test Type (Flow-through, Static, Static Renewal): Flow-through

NOAEC: 49 µg ai/L
LOAEC: 95 µg ai/L

Endpoint(s) affected: growth (standard length and dry weight) and clinical signs of toxicity
Most sensitive endpoint(s): growth (standard length and dry weight)

I. MATERIALS AND METHODS

GUIDELINE(S) FOLLOWED: This study was conducted following guidelines outlined in the U.S. EPA Ecological Effects Test Guideline 850.1400: *Fish Early-Life Stage Toxicity Test* (draft, 1996); U.S. EPA Pesticide Assessment Guidelines, §72-4(a) (1986) and associated Standard Evaluation Procedure, Fish Early Life-Stage, EPA-540/9-86-138 (1985); and OECD Test Guideline 210, Fish, Early-life stage toxicity test (1992).

Deviations from OCSPP 850.1400 guidance included:

1. The age of the embryos (24- to 48-hours old) slightly exceeded recommendations (2- to 24-hours old).

This deviation does not affect the acceptability of this study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality claims statements were provided. This study was conducted in accordance with GLP Standards as published in 40 CFR Part 160 with the following exception: routine water contaminant screening analyses.

A. MATERIALS:

1. Test Material Flufenacet Technical

Description: Solid, light beige color

Lot No./Batch No. : NK61CX0617

Purity: 98.83%

Stability of compound under test conditions: Stable, as determined from weekly analyses of test solutions at all treatment levels. For all levels, coefficients of variation (CV) were 3.37 to 5.64%.

Storage conditions of Test chemicals: Ambient

Physicochemical properties of flufenacet.

Parameter	Values	Comments
Water solubility at 20°C	Not reported	See range-finding study section below.
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

2. Test organism:

Species:	Sheepshead minnow (*Cyprinodon variegatus*) [*EPA recommends any of several freshwater fish species, including rainbow trout, brook trout, bluegill, fathead minnow, and channel catfish. See Standard Evaluation Procedure for listing of recommended species. OECD recommends rainbow trout, fathead minnows, zebra fish, and ricefish but does not exclude the use of other species.*]
Age/embryonic stage at test initiation:	Embryos (Lot No. ABS052413), 24- to 48-hours old in the neurula stage (determined via a light microscope) [*EPA recommends fish embryos 2 to 24 hours old.*]
Method of collection of the fertilized eggs:	N/A (purchased)
Source:	Aquatic Biosystems, Fort Collins, CO

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: No preliminary range-finding study was conducted; nominal test concentrations were determined based on historical data.

A preliminary solubility trial indicated that flufenacet was soluble in dilution water at 20 mg ai/L (85% recovery) and stable in solution for at least 7 days.

Two definitive study attempts were performed. The first attempt was conducted between November 9 to December 14, 2012 at nominal test concentrations of 0 (negative and solvent controls), 50, 100, 200, 400 and 800 µg ai/L. This study was cancelled after study completion due to variability between the control and solvent control data. Comprehensive data were collected and provided in the study report. The second definitive attempt was performed from May 17-21, 2013 at nominal concentrations of 0 (negative control), 50, 100, 200, 400 and 800 µg ai/L; the study was cancelled on Day 4 due to low analytical recoveries on Day 0.

c. Definitive study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental acclimation, if any Period: Conditions (same as test or not): Feeding (type, source, amount given, frequency): Health: (any mortality observed)	N/A	Upon receipt, embryos were placed in dilution water and acclimated from 20.1 to 25°C (test temperature) over a 28-minute period. Microscopic examinations were made to determine the stage of development, and eggs that did not appear healthy were not used.
Number of fertilized eggs/embryos in each treatment at test initiation	Initial: 35 embryos per replicate x 4 replicates per level (140 embryos per level) Following hatch: 20 alevins per replicate x 4 replicates per level (80 alevins per level)	Eggs were impartially selected. *Each treatment should include a minimum of 20 embryos per replicate cup and a minimum of 30 fish per treatment for post-hatch exposure (OECD recommends at least 60 eggs, divided between at least 2 replicates)*

Parameter	Details	Remarks *Criteria*
Concentration of test material nominal: mean measured:	0 (negative control), 50, 100, 200, 400 and 800 µg ai/L <4.0 (<LOQ, control), 49, 95, 174, 339 and 677 µg ai/L	For concentration verification, samples were collected from two alternating replicate chambers on Days 0, 7, 14, 21, 28 and 35. Samples were stabilized upon collection with 1.0% formic acid to prevent degradation during sample transport and analysis. Concentrations were satisfactorily maintained, with coefficients of variation (CV) of 3.37 to 5.64% for all levels. Mean-measured concentrations represented 85 to 98% of nominal levels. *A minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate should be used.* *- Toxicant concentration should be measured in one tank at each toxicant level every week.* *- One concentration should adversely affect a life stage and one concentration should not affect any life stage.* *OECD recommends that 5 concentrations be spaced by a constant factor not exceeding 3.2; concentrations of test substance in solution should be within ± 20% of the mean measured values.*
Solvent (type, percentage, if used)	N/A	*The solvent should not exceed 0.1 ml/L in a flow-through system. Recommended solvents include dimethylformamide, triethylene glycol, methanol, acetone, ethanol.* *OECD recommends that the solvent not have an effect on survival nor produce any other adverse effects;concentration should not be greater than 0.1 ml/L.*
Number of replicates control: solvent control: treated ones:	4 N/A 4/level	*Number of replicates should be 4 per concentration.* *A solvent control should be used in conjunction with a solubilizing agent.*

Parameter	Details	Remarks *Criteria*
Test condition static renewal/flow-through: type of dilution system for flow through method: flow rate: renewal rate for static renewal:	 Flow-through Intermittent-flow proportional diluter *ca.* 7 volume additions per day N/A	The exposure system was properly operating for *ca.* 3 days prior to test initiation to allow equilibration of the test substance in the diluter apparatus and aquaria. The splitting accuracy (±10%) of the delivery system was calibrated prior to test initiation. Proper operation of the diluter system was checked at least once daily during the study. *Intermittent flow proportional diluters or continuous flow serial diluters should be used. EPA recommends that flow rate to larval cups should provide 90% replacement in 8 to 12 hours (OECD recommends 5 test chamber volumes/24 hours). For static-renewal, OECD recommends 2 renewal procedures; either transfer eggs and larvae to new, clean vessels or retain organisms in vessels and change at least 2/3 test water. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used.* *Toxicant Mixing:* *1) Mixing chamber is preferred;* *2) Aeration should not be used for mixing;* *3) The test solution should be completely mixed before introduction into the test system;* *4) Flow splitting accuracy should be within 10%.*
Aeration, if any	No supplemental aeration was used.	 *Dilution water should be aerated to ensure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.*
Duration of the test	35 days: 6-day hatching period and 29-day post-hatch period	Acceptable for this species under OCSPP guidance. *Recommended test duration is 32 days for EPA. OECD recommendations for test duration are species specific and range from 28-60 days.*

Parameter	Details	Remarks *Criteria*
<u>Embryo cups, if used</u> type/material (glass/stainless steel): size: fill volume:	Not reported	Embryo cups were oscillated. *Recommended embryo cups are 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.*
<u>Test vessel</u> type/material: (glass/stainless steel) size: fill volume:	Glass 8.4 L (21.6 x 12.7 x 30.5 cm) 7 L (21.6 x 12.7 x 25.5 cm)	 *Recommended test vessel is all glass or glass with stainless steel frame.*
Source of dilution water	Dilution water was prepared by mixing artificial sea salts (Crystal Sea MarineMix from Marine Enterprises International) with process water to a salinity of 20 ± 5‰. Process water consisted of spring water that had been blended with reverse-osmosis (de-chlorinated) tap water. Both water sources were subjected to various filtrations and sterilization procedures prior to blending. The automated system constantly monitored the municipal water after de-chlorination. Blended water was stored in polypropylene or PVC holding tanks and intensely aerated before use.	Based upon weekly and/or monthly screens of the dilution water: total suspended solids <1.0 mg/L, unionized ammonia <0.010 mg/L, and total residual chlorine <0.003 mg/L. Results from the April 2013 bi-annual screening of each water source for various organic and inorganic contaminants were also provided. *Source of dilution water should be natural or reconstituted water; natural water should be sterilized with UV and tested for pesticides, heavy metals, and other possible contaminants.* *OECD accepts any water in which the test species show control survival at least as good as presented in SEP.*

Parameter	Details	Remarks *Criteria*
Water parameters hardness: pH: dissolved oxygen: temperature (s) (record all the temperatures used for different life stages): photoperiod: salinity (for marine or estuarine species): other measurements: interval of water quality measurements:	 Not applicable 8.1 to 8.2 5.3 to 7.5 mg/L (70 to 99% saturation) 24.7 to 25.5°C (mean 25.1°C) 16-hr light:8-hr dark photoperiod (with 30-minute transition periods); 736 to 805 lux 18 to 22‰ (mean 20‰) N/A DO, pH, and salinity were measured in all levels at study initiation and weekly thereafter in all levels. Temperature was recorded hourly in a centrally-located test vessel.	Light intensity was measured once prior to experimental start at the surface of the test solutions. *Recommended hardness: 40-48 mg/L as $CaCO_3$;* *Recommended pH:* 7.2 to 7.6 *Dissolved Oxygen (DO) should be measured at each concentration at least once a week;* *Freshwater parameters in a control and one concentration should be analyzed once a week.* *Temperature depends upon test species and should not deviate by more than 2°C from appropriate temperature.* *OECD recommends that DO concentration be between 60 - 90% saturation. As a minimum DO, salinity (if relevant) and temperature should be measured weekly, and pH and hardness at the beginning and end of the test. Temperature should be measured continuously.*
Post-hatch details when the post-hatch period began: number of hatched eggs (alevins)/ treatment released to the test chamber: on what day, the alevins were released from the incubation cups to the test chamber:	 Day 6 20 alevins per replicate (80 per level) Day 6	OSCPP specifies a control hatching success criterion of >75% and a post-hatch survival of ≥80%. Both validity criteria were met. *Percentage of embryos that produce live fry should be ≥ 50% in each control; percentage of hatch in any control embryo cup should not be more than 1.6 times that in another control cup.*
Post-hatch Feeding start date: type/source of feed: amount given: frequency of feeding:	 Day 5 24- to 48-hour old live brine shrimp nauplii (*Artemia salina*) 0.5 to 4.0 mL per feeding, increasing as the fish grew Two to three times daily	The fish were not fed during the final 24 hours prior to test termination.

Parameter	Details	Remarks *Criteria*
Recovery of chemical: Frequency of measurement: LOD: LOQ:	96 to103% of nominal Days 0, 7, 14, 21, 28 and 35 Not reported 0.5 µg ai/L	Based on concurrently-run procedural recovery (QC) samples fortified at 50.0 ppb and analyzed with each sample set.
Positive control {if used, indicate the chemical and concentrations}	N/A	
Fertilization success study, if any number of eggs used: on what day the eggs were removed to check the embryonic development:	N/A	
Other parameters, if any	Biomass loading was 0.096 g/L/day	Based on mean wet weight of control fish (0.23 g/fish) at study termination. Within OPPTS requirements.

2. Observations:

Table 2: Observations

Parameters	Details	Remarks *Criteria*
Parameters measured including the sub-lethal effects/toxicity symptoms	- Time to hatch - Hatching success - Post-hatch survival - Measurement of growth (standard length and dry weight) - Morphological and behavioral effects	*Recommended parameters measured include:* *- Number of embryos hatched;* *- Time to hatch;* *- Mortality of embryos, larvae, and Juveniles:* *- Time to swim-up (if appropriate);* *- Measurement of growth;* *- Incidence of pathological or Histological effects;* *- Observations of other effects or clinical signs.*

Parameters	Details	Remarks *Criteria*
Observation intervals/dates for: egg mortality: no. of eggs hatched: mortality of fry (e.g.,alevins): swim-up behavior: growth measurements: embryonic development: other sub-lethal effects	 Daily Daily Daily N/A Day 35 Daily Daily	
Water quality was acceptable (Yes/No)	Yes	
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY:

Alevin survival was assessed on Day 6 and ranged from 87.9 to 90.7% for all levels, with no statistically-significant differences from the control indicated for any level. Fry survival was assessed on Day 35 and ranged from 95.0 to 98.8%, with no statistically-significant differences from the control indicated for any level. The NOAEC and LOAEC for alevin and fry survival were 667 and >667 µg ai/L, respectively.

Table 3: Effect of flufenacet on egg hatching and survival at different life stage of fish.

Treatment (µg ai/L) Mean-measured (and nominal) concentrations	No. of initial eggs	Cumulative % Hatch ± SD (and % Inhibition)		% Survival ± SD (and % Inhibition)	
		Day 5	Day 6	Day 6[(a)]	Day 35
Negative Control	140	37.9 ± 21.8	85.7 ± 2.3	90.7 ± 2.7	98.8 ± 2.5
49 (50)	140	36.4 ± 12.4 (3.77)	87.1 ± 4.9 (-1.67)	87.9 ± 5.9 (3.1)	96.3 ± 2.5 (2.5)
95 (100)	140	39.3 ± 8.5 (-3.77)	84.3 ± 8.6 (1.67)	90.0 ± 4.9 (0.8)	98.8 ± 2.5 (0.0)
174 (200)	140	35.0 ± 15.2 (7.55)	86.4 ± 9.7 (-0.83)	90.0 ± 4.9 (0.8)	98.8 ± 2.5 (0.0)
339 (400)	140	38.6 ± 15.2 (-1.89)	87.1 ± 5.5 (-1.67)	90.7 ± 4.9 (0.0)	97.5 ± 2.9 (1.3)
677 (800)	140	29.3 ± 15.9 (22.64)	89.3 ± 4.3 (-4.17)	89.3 ± 4.3 (1.6)	95.0 ± 4.1 (3.8)
NOAEC, µg ai/L		667			
LOAEC, µg ai/L		>667			
EC_{50}		>667			
Positive control, if used		N/A			

[(a)] Prior to thinning [to 20 per replicate (80 per level)] at the completion of hatch on Day 6.
* Statistically significant difference compared to the negative control (p=0.05).

B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:

Time to hatch: The time to hatch was assessed by comparison of the percentages of hatched minnow on Days 5 and 6. No treatment-related effect on time to hatch was observed. On Days 5 and 6, percent hatch for all levels ranged from 29.3 to 39.3% and 84.3 to 89.3%, respectively, no statistically-significant differences from the control indicated at any level or interval. The NOAEC and LOAEC for time to hatch were 677 and >677 µg ai/L, respectively.

Clinical signs of toxicity: With the exception of a few small-sized fish noted throughout various test levels, fish from the control through mean-measured 339 µg ai/L treatment levels appeared normal during the study. At the 677 µg ai/L level, fish were observed to be swimming at the bottom of the test vessel, except when being fed, beginning on Day 32. In addition, one fish from the 174 µg ai/L was noted with a blunt snout at termination, which was considered to be incidental to exposure. The NOAEC and LOAEC for clinical signs of toxicity were 339 and 677 µg ai/L, respectively.

Growth: At study termination, fish were sacrificed and measured for standard length and dry weight. Growth was the most sensitive endpoint. For the mean-measured 0 (control), 49, 95, 174, 339 and 677 µg ai/L treatment levels, standard lengths averaged 19.4, 19.3, 19.0, 18.8, 18.6 and 18.1 mm, respectively, and dry weights averaged 62.7, 61.9, 58.1, 58.8, 56.9 and 50.8 mg, respectively. For both growth parameters, differences were statistically-significant compared to the control (p=0.05) at the ≥95 µg ai/L levels. The subsequent NOAEC and LOAEC for growth were 49 and 95 µg ai/L, respectively.

Table 4: Effect of flufenacet on growth of juvenile fish.

Treatment (µg ai/L) Mean-measured (and nominal) concentrations	Growth Day 35 (29 days post-hatch)	
	Mean Standard Length, mm ± SD (and % Inhibition)	Mean Dry Weight, g ± SD (and % Inhibition)
Negative Control	19.4 ± 0.13	62.7 ± 1.07
49 (50)	19.3 ± 0.22 (0.64)	61.9 ± 2.48 (1.15)
95 (100)	19.0 ± 0.17 (2.01)*	58.1 ± 1.07 (7.22)*
174 (200)	18.8 ± 0.18 (2.91)*	58.8 ± 1.93 (6.15)*
339 (400)	18.6 ± 0.35 (4.00)*	56.9 ± 2.98 (9.15)*
677 (800)	18.1 ± 0.13 (6.89)*	50.8 ± 0.38 (19.02)*
NOAEC, µg ai/L	49	
LOAEC, µg ai/L	95	

* Statistically significant difference compared to the negative control (p=0.05).

C. REPORTED STATISTICS:

Statistical Method: Data that were statistically analyzed included survivorship (alevin and fry), hatchability (time to hatch), and growth (length and dry weight). For each parameter, the data were tested for normality using the Chi-square test and for homogeneity of variance using Barlett's test. Data for all parameters met these assumptions and were subsequently analyzed using ANOVA followed by Dunnett's test and (if appropriate) William's test.

Analyses were performed using TOXSTAT (v. 3.4). The NOAEC and LOAEC were based on significance data. Results were reported in terms of mean-measured concentrations.

NOAEC: 49 µg ai/L
LOAEC: 95 µg ai/L

Endpoint(s) affected: growth (standard length and dry weight)
Most sensitive endpoint(s): growth (standard length and dry weight)

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): The reviewer analyzed hatching success, larval survival, post-hatch survival, mean length, and mean dry weight. All data were tested for normality using the Shapiro-Wilk's test and for homogeneity of variance using Bartlett's test. All endpoints except larval survival met the assumptions of parametric statistics and were therefore analyzed using Dunnett's test. Larval survival was analyzed using the Mann-Whitney U Two-Sample Test. Mean length and mean dry weight also exhibited suggestive monotonically decreasing trends and were therefore also analyzed using Williams' test. All analyses were conducted using CETIS version 1.8.7.12 with database backend settings implemented by EFED on 3/25/14. Tests for normality and homogeneity of variance were conducted at $\alpha = 0.01$, whereas all other tests were conducted at $\alpha = 0.05$. All analyses were conducted using the mean-measured concentrations.

Hatching Success:
NOAEC: 677 µg ai/L
LOAEC: >677 µg ai/L

Larval Survival (Day 6):
NOAEC: 677 µg ai/L
LOAEC: >677 µg ai/L

Post-Hatch Survival (Day 35):
NOAEC: 677 µg ai/L
LOAEC: >677 µg ai/L

Mean Length:
NOAEC: 49 µg ai/L
LOAEC: 95 µg ai/L

Dry Weight:
NOAEC: 49 µg ai/L
LOAEC: 95 µg ai/L

E. STUDY DEFICIENCIES:

There were no deficiencies affecting the scientific soundness or acceptability of this study. One deviation from U.S. EPA OPPTS guidance was observed: the study was initiated with *ca.* 24- to 48-hour old embryos, whereas guidance recommends the use of 2- to 24-hour old embryos.

F. REVIEWER'S COMMENTS:

The reviewer's statistical conclusions agreed with those reported by the study authors. The reviewer's mean values for the various survival endpoints differed slightly from those calculated by the study author. These differences were due to the way these values were calculated. For example, larval survival was calculated by the study authors based on the total number of live alevins and unhatched eggs, whereas the reviewer only included live alevins. These differences were minor and had no influence on the statistical findings. Results were provided in terms of mean-measured concentrations.

Stock solutions were prepared as needed once every 6 to 7 days at a nominal concentration of 20 mg ai/L in dilution water. The stock solutions were sonicated for a minimum of 9 hours. While not in use, stock solutions were stored at room temperature.

Aliquots (1.0 mL) of the exposure and QC samples were partitioned three times with methyl-t-butyl ether (MTBE) while vortexing. The organic phases were combined following each extraction, and the samples were evaporated to dryness. Residues were reconstituted in 2.0 mL of acetonitrile:water (1:1, v:v) containing 0.1% formic acid and mixed well. Aliquots were analyzed for flufenacet using HPLC-MS/MS. The analytical limit of quantification (LOQ) was 4.0 µg ai/L.

The in-life phase of the definitive study was conducted from May 24 to June 28, 2013.

G. CONCLUSIONS:

This study **is scientifically sound** and is classified as **acceptable**. Based on treatment-related reductions in Day-35 standard length and dry weight at the ≥95 µg ai/L treatment levels, the NOAEC and LOAEC for the study were 49 and 95 µg ai/L, respectively. In addition, treatment-related signs of stress (swimming at the bottom of the vessel) were observed in fish from the 677 µg ai/L level. There were no treatment-related effects at any exposure level for alevin survival prior to thinning (on Day 6), fry survival following thinning, percent hatch, or time to hatch.

NOAEC: 49 µg ai/L
LOAEC: 95 µg ai/L

Endpoint(s) affected: growth (standard length and dry weight) and clinical signs of toxicity
Most sensitive endpoint(s): growth (standard length and dry weight)

III. REFERENCES:

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OPPTS 850.1400 Chronic Fish Early Life Stage (ELS) **SynTech Research Laboratory Services LLC**

Batch ID:	09-9386-0175	**Test Type:**	Fish ELS (28-60d) Test	**Analyst:**	
Start Date:	24 May-13	**Protocol:**	OPPTS 850.1400 Chronic Early Life Stage	**Diluent:**	Reverse Osmosis Water
Ending Date:		**Species:**	Cyprinodon variegatus	**Brine:**	Crystal Sea
Duration:	NA	**Source:**	Aquatic Biosystems, CO	**Age:**	<48h
Sample ID:	07-6848-6400	**Code:**	49244201	**Client:**	CDM Smith
Sample Date:	24 May-13	**Material:**	Flufenacet	**Project:**	Herbicide
Receive Date:		**Source:**	Bayer CropScience AG		
Sample Age:	NA	**Station:**			

Batch Note: PC Code 121903 MRID 49244201
Sample Note: PC Code 121903 MRID 49244201

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
00-1444-2289	Hatching Success	677	>677	NA	12.6%		Dunnett Multiple Comparison Test
16-7150-9470	Larval Survival	677	>677	NA	0.94%		Mann-Whitney U Two-Sample Test
04-6926-3630	Mean Dry Weight	49	95	68.23	5.1%		Dunnett Multiple Comparison Test
12-4982-0471	Mean Dry Weight	49	95	68.23	3.96%		Williams Multiple Comparison Test
05-4566-6868	Mean Length	49	95	68.23	1.82%		Dunnett Multiple Comparison Test
02-2207-6067	Mean Length	49	95	68.23	1.41%		Williams Multiple Comparison Test
06-9829-5726	Post Hatch Survival	677	>677	NA	4.98%		Dunnett Multiple Comparison Test

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Hatching Success Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	0.857	0.82	0.894	0.829	0.886	0.0117	0.0233	2.72%	0.0%
49		4	0.871	0.793	0.95	0.829	0.914	0.0247	0.0495	5.68%	-1.67%
95		4	0.836	0.705	0.966	0.771	0.943	0.041	0.0821	9.82%	2.5%
174		4	0.864	0.71	1	0.743	0.943	0.0486	0.0972	11.3%	-0.83%
339		4	0.871	0.784	0.958	0.829	0.943	0.0274	0.0547	6.28%	-1.67%
677		4	0.893	0.825	0.961	0.857	0.943	0.0214	0.0429	4.8%	-4.17%

Larval Survival Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	0.992	0.965	1	0.967	1	0.00833	0.0167	1.68%	0.0%
49		4	1	1	1	1	1	0	0	0.0%	-0.84%
95		4	1	1	1	1	1	0	0	0.0%	-0.84%
174		4	1	1	1	1	1	0	0	0.0%	-0.84%
339		4	1	1	1	1	1	0	0	0.0%	-0.84%
677		4	1	1	1	1	1	0	0	0.0%	-0.84%

Mean Dry Weight Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	62.7	60.9	64.4	61.3	63.9	0.542	1.08	1.73%	0.0%
49		4	62	58	65.9	58.9	64.9	1.24	2.48	4.01%	1.16%
95		4	58.2	56.4	59.9	56.7	59.3	0.538	1.08	1.85%	7.22%
174		4	58.8	55.7	61.9	56.6	60.7	0.964	1.93	3.28%	6.18%
339		4	57	52.2	61.7	53.4	60.3	1.49	2.97	5.22%	9.13%
677		4	50.8	50.2	51.3	50.2	51	0.185	0.37	0.73%	19.0%

Mean Length Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	19.4	19.2	19.6	19.3	19.6	0.0629	0.126	0.65%	0.0%
49		4	19.3	19	19.6	19.1	19.6	0.108	0.216	1.12%	0.64%
95		4	19	18.7	19.4	18.8	19.2	0.103	0.206	1.08%	2.06%
174		4	18.8	18.6	19.1	18.6	19	0.0854	0.171	0.91%	3.09%
339		4	18.7	18.1	19.2	18.2	19	0.166	0.332	1.78%	3.99%
677		4	18.1	17.9	18.3	17.9	18.2	0.0629	0.126	0.7%	6.95%

Post Hatch Survival Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	0.988	0.948	1	0.95	1	0.0125	0.025	2.53%	0.0%
49		4	0.962	0.923	1	0.95	1	0.0125	0.025	2.6%	2.53%
95		4	0.988	0.948	1	0.95	1	0.0125	0.025	2.53%	0.0%
174		4	0.988	0.948	1	0.95	1	0.0125	0.025	2.53%	0.0%
339		4	0.975	0.929	1	0.95	1	0.0144	0.0289	2.96%	1.27%
677		4	0.95	0.885	1	0.9	1	0.0204	0.0408	4.3%	3.8%

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Hatching Success Detail

C-µg ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	0.857	0.857	0.886	0.829
49		0.829	0.914	0.914	0.829
95		0.771	0.771	0.857	0.943
174		0.829	0.743	0.943	0.943
339		0.886	0.943	0.829	0.829
677		0.857	0.943	0.857	0.914

Larval Survival Detail

C-µg ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	1	0.967	1	1
49		1	1	1	1
95		1	1	1	1
174		1	1	1	1
339		1	1	1	1
677		1	1	1	1

Mean Dry Weight Detail

C-µg ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	61.3	63	62.5	63.9
49		64.9	62.5	58.9	61.5
95		58.3	59.3	58.3	56.7
174		60.1	60.7	56.6	57.8
339		58.2	55.9	53.4	60.3
677		50.9	51	50.2	50.9

Mean Length Detail

C-µg ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	19.6	19.4	19.3	19.4
49		19.6	19.3	19.2	19.1
95		19.2	19.2	18.9	18.8
174		19	18.9	18.8	18.6
339		19	18.7	18.2	18.7
677		18.1	18.2	17.9	18.1

Post Hatch Survival Detail

C-µg ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	1	0.95	1	1
49		0.95	0.95	1	0.95
95		1	0.95	1	1
174		1	1	0.95	1
339		1	0.95	1	0.95
677		0.95	0.9	0.95	1